Protocol ID: 853701

Title: Erbium, Chromium: Yttrium, Scandium, Gallium, Garnet (Er,Cr:YSGG) Laser in Root Canal Disinfection

NCT05959629

5/9/2023

CLINICAL RESEARCH PROTOCOL

INVESTIGATIONAL PRODUCT(S):		Waterlase Express™, BIOLASE®. Root canal disinfection protocol
STUDY NUMBER(S):	**IRB Number**	
	Other Protocol Identifiers	N/A
PROTOCOL(S) TITLE:		A clinical use of Er,Cr:YSGG laser: an anti-biofilm treatment
REGULATORY SPONSOR:		Penn Dental Medicine – Endodontic Department Evans Building 4001 Spruce St Philadelphia, PA 19104-6003
FUNDING SPONSOR(S):		Penn Dental Medicine – Endodontic Department
MEDICAL DIRECTOR		Dr. Bekir Karabucak (Chair of Endodontic Department)
ORIGINAL PROTOCOL DATE:		
VERSION NUMBER:		V1.0
VERSION DATE:		05.09.2023

PRINCIPAL INVESTIGATOR SIGNATURE

STUDY SPONSOR:	Penn Dental Medicine – Endodontic Department
STUDY TITLE:	A clinical use of Er,Cr:YSGG laser: an anti-biofilm treatment
STUDY ID	[Insert IRB #]
PROTOCOL VERSION	V1.0

I have read the referenced protocol. I agree to conduct the study in accordance to this protocol, in compliance with the Declaration of Helsinki, Good Clinical Practices (GCP), and all applicable regulatory requirements and guidelines.

Principal Investigator Name	Dr. Bekir Karabucak/Dr. Flavia Teles	Signature	Flavia Teles
Affiliation:	Penn Dental Medicine	Date	05/09/2023

Abbreviations

AE	Adverse Event
ANCOVA	Analysis of Covariance
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
MP	Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization

ITT	Intention-To-Treat
LSMEANS	Least-Squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
DSMC	Data Safety Monitoring Committee
SoA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
UADE	Unanticipated Adverse Device Effect
UP	Unanticipated Problem
US	United States

1 STUDY SUMMARY

1.1 Synopsis

Title:	A clinical use of Er,Cr:YSGG laser: an anti-biofilm treatment
Short Title:	Er,Cr:YSGG laser in Endodontics
Study Description:	The purpose of this study is to develop a protocol for biofilms disinfection with a clinically approved and commercially available Er,Cr:YSGG laser treatments. This protocol will be testing local single topical application of Lasers within the canal system in patients going through routine endodontic treatment, evaluate its potential as anti-biofilm treatment and compare it to other currently used antibacterial protocols.
Objectives:	The purpose of this study is to develop a better, more effective disinfection protocol against biofilms within the root canal system, subsequently, improve the success of root canal treatment in teeth with apical periodontitis. This protocol will be testing local single topical application of a very low dose of 2780 nm Er,Cr:YSGG laser within the canal system in patients going through routine endodontic treatment, will evaluate its potential as an advanced antibacterial, anti-biofilm treatment by intra-canal bacterial sampling and compare it to other currently used antibacterial protocols.
Primary Endpoint:	The difference in bacterial reduction between the experimental group (laser) and the comparison group (routinely used irrigation protocol) by measuring reduction in colony forming units (CFU). Friedman's and Wilcoxon signed-rank tests will be used to compare the amount of bacteria before and after treatment in both groups, with significance levels set at 5% ($P < 0.05$).
Secondary Endpoints:	Secondary endpoints: - **Measurement of pain:** Following a previously published study conducted at the Department of Endodontics, University of Pennsylvania[1]. Patients will be asked to rate the intensity of preoperative pain on a numeric rating scale (NRS) from 0 (no pain) to 10 (worst pain) before receiving root canal treatment. Along with NRS, the Wong-Baker facial grimace scale will also presented to the patients to help them in scoring the pain. At the end of each visit, the patients will be given a survey and asked to rate the

intensity of postoperative pain at 4, 24, and 48 h after the procedure. Patients will be instructed to take 1000-mg acetaminophen as needed. If acetaminophen was consumed, the patients will be asked to record the dose and time on the survey. They will be provided stamped return envelopes to mail surveys back to the Department of Endodontics *(attachment 1).*

- **Treatment success determined clinically and radiographically:** Radiographically, Following periapical (PAI) index by Örstavik[2], description of radiographic fndings: 1. Normal periapical structures. 2. Small changes in the bone structure. 3. Change in the bone structure with mineral loss. 4. Periodontitis with a well-defined radiolucent area. 5. Severe periodontitis with exacerbating features. Success is defined as either complete (radiographic resolution of a periapical lesion - the radiographic sign of inflammatory processes surrounding a root tip) or incomplete healing (scar tissue formation) and failure includes uncertain healing (radiographic reduction of a periapical lesion or same lesion size) or unsatisfactory healing (increase in lesion size) as determined on the radiograph. Clinically, success is defined by the absence of pain, swelling, percussion sensitivity or sinus tracts. Clinical failure is defined as the persistent presence of any of the symptoms mentioned above. Treatment success/failure measured at the standard of care 6 month, 1 year and 2 year follow up (± 7 days) post root canal filling.

Study Population:	76 Patients presenting to the Department of Endodontics, School of Dental Medicine, University of Pennsylvania for evaluation and routine endodontic treatment of infected, necrotic teeth with chronic apical periodontitis.
Phase:	Postmarket
Description of Sites/Facilities	Department of Endodontics, School of Dental Medicine, University of Pennsylvania.
Enrolling Sites:	Patients presenting to the Department of Endodontics, School of Dental Medicine, University of Pennsylvania for evaluation and routine endodontic treatment of infected, necrotic teeth with chronic apical periodontitis.

Description of Study Intervention:	An ER:Cr;YSSG laser 2780 nm (Waterlase Express™, BIOLASE®) with 300µm tip (EdgePro #3, RFT 3) will be placed into the mid-root of the canal. The tip will be activated and slowly withdrawn to the orifice (1-2mm/sec) following the manufacturer settings (energy 15mJ, repetition rate 50Hz, 0% air, 0% water).
Study Duration:	The projected time frame for this research project is from June 2023 to June 2027. Enrollment is from June 2023 to June 2025. Followed by 2 years for the last follow up, which is the date of the second visit for the last subject enrolled +2 years (± 7 days). The recruitment, treatment and analysis of 76 subjects with teeth matching the inclusion criteria can be reasonably achieved within these 4 years.
Participant Duration:	The subjects will participate in the study for 2 years. This is the standard of care timeline for routine root canal therapy with 6 months, 1 year, and 2 year follow up.

1.2 Key Roles and Study Governance

Sponsor	**Medical Director**
Endodontic Department	Bekir Karabucak, DMD, MS. Chair and Professor of Endodontics, Postdoctoral Endodontics Program, Director.
Penn Dental Medicine	Penn Dental Medicine
Evans Building 4001 Spruce St Philadelphia, PA 19104-6003	Evans Building, 3rd floor 4001 Spruce St Philadelphia, PA 19104-6003
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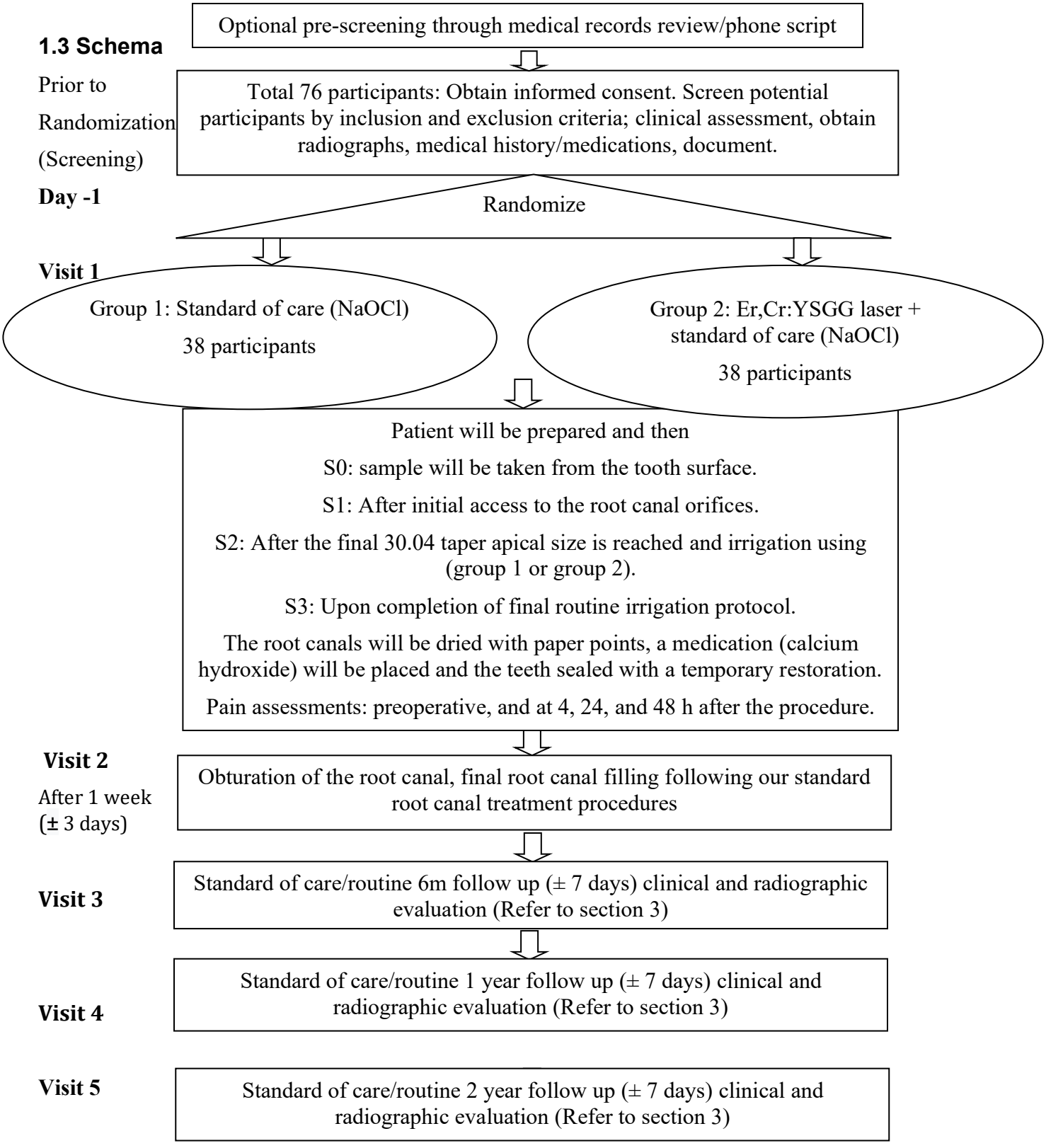
1.3 Schema


Optional pre-screening through medical records review/phone script
Prior to Randomization (Screening)
Total 76 participants: Obtain informed consent. Screen potential participants by inclusion and exclusion criteria; clinical assessment, obtain radiographs, medical history/medications, document.
Day -1
Randomize
Visit 1
Group 1: Standard of care (NaOCl)
38 participants
Group 2: Er,Cr:YSGG laser + standard of care (NaOCl)
38 participants
Patient will be prepared and then
S0: sample will be taken from the tooth surface.
S1: After initial access to the root canal orifices.
S2: After the final 30.04 taper apical size is reached and irrigation using (group 1 or group 2).
S3: Upon completion of final routine irrigation protocol.
The root canals will be dried with paper points, a medication (calcium hydroxide) will be placed and the teeth sealed with a temporary restoration.
Pain assessments: preoperative, and at 4, 24, and 48 h after the procedure.
Visit 2
After 1 week (± 3 days)
Obturation of the root canal, final root canal filling following our standard root canal treatment procedures
Visit 3
Standard of care/routine 6m follow up (± 7 days) clinical and radiographic evaluation (Refer to section 3)
Visit 4
Standard of care/routine 1 year follow up (± 7 days) clinical and radiographic evaluation (Refer to section 3)
Visit 5
Standard of care/routine 2 year follow up (± 7 days) clinical and radiographic evaluation (Refer to section 3)

Visit 1 Sampling Protocol

* Before all samplings, solutions will be removed with 5cc of sterile saline and canal content will be neutralized with 5% sodium thiosulfate solution.

Step	Followed by*
Rubber dam application. Chlorhexidine 2% will be used to disinfect the tooth and the rubber dam. The removal of caries and the endodontic access will be carried out by sterile high-speed carbide burs. Chlorhexidine 2% will be used to disinfect the tooth and the rubber dam again.	**Sample 0**
After initial access to the root canal orifices, a bacteriological sample will be taken from the targeted canals.	**Sample 1**
Canals will be instrumented up to size 30/0.04 taper using either (NaOCl) or (laser). (Refer to study design section 4.1)	**Sample 2**
All canals in all groups will be irrigated with 5cc of 3% NaOCl with the ultrasonic irrigant activation following the standard endodontic procedure protocol.	**Sample 3**

2 INTRODUCTION AND RATIONALE

2.1 Study Rationale

The purpose of this study is to develop a better, more effective disinfection protocol against biofilms within the root canal system, subsequently, improve the success of root canal treatment in teeth with apical periodontitis. This protocol will be testing local single topical application of a very low dose of an ER:Cr;YSSG laser 2780 nm (Waterlase Express™, BIOLASE®) within the canal system in patients going through routine endodontic treatment, will evaluate its potential as an advanced antibacterial, anti-biofilm treatment by intra-canal bacterial sampling and compare it to other currently used antibacterial protocols.

2.2 Background

Endodontic research has established the importance of microorganisms in the pathogenesis of apical periodontitis[3-5]. The reduction or elimination of microorganisms is a logical aim for successful endodontic treatment. Sjörgen et al[6] evaluated the long-term outcome after one step endodontic treatment of teeth with negative or positive cultures after instrumentation. 94% of the teeth filled after a negative culture succeeded, whereas only 68% of the canals filled after a positive culture had been detected were successful. Based on this study the optimal clinical success will be reached if a tooth is filled when the bacterial load of the canal is undetectable with culturing methods. Because culturing is as yet only effective as a research tool, the clinician should expect optimal success when treating an infected tooth with a technique or protocol that has been shown experimentally to result in consistent negative cultures. Byström et al,[7-9] published a series of studies to evaluate the antibacteriological effects of the individual steps in the endodontic procedure. Only after instrumentation using endodontic instruments with NaOCl and EDTA irrigation and a dressing of calcium hydroxide it was possible to obtain a predictable negative culture[7-9]. However, Histo-bacteriological studies have shown that even after meticulous chemo-mechanical preparation using endodontic instruments with NaOCl and EDTA and obturation of the root canal system, a significant amount of bacterial biofilm is often present in the anatomical complexities of the root canal system (isthmuses, apical ramification, accessory canals, and dentinal tubules) and on the walls of the main root canal that was not engaged by the root canal instruments[10-12].

Endodontic biofilms may play a key role in infections persisting after primary or secondary endodontic treatment, as microorganisms growing in biofilms are better protected from adverse environmental changes and other antimicrobial agents. Ricucci et al,[13] evaluated the prevalence of bacterial biofilms in untreated root canals of teeth showing apical periodontitis in 80% of the cases. Nevertheless, the persistence of endodontic infections is also attributed to the presence of the aforementioned anatomical complexities, which can harbor biofilm and protect it from the effects of the current disinfection solutions[14]. Based on these facts, advanced disinfection strategies with the ability to disrupt biofilm are needed to achieve the goal of endodontic treatment and to

overcome the persistence of endodontic infections, if possible, using less harsh chemicals as currently used (e.g. NaOCl).

The great evolutionary leap in solving this problem is the association of biomechanical preparation with mechanized instrumentation, including abundant ultrasonic irrigation of antiseptic solutions associated with surfactants, laser ablation, and three-dimensional filling of the root canal system[15]. For this evolutionary leap to be fully achieved, all clinical steps, that is, instrumentation, irrigation, ablation, and filling, must be used, since the absence of one of them makes the complete disinfection of the root canal system unfeasible. The literature demonstrates the limitations of irrigating solutions, instrumentation and the partial effect of the action of ultrasound, resulting in a filling in a still infected medium. In addition to this, during the instrumentation, a contaminated residual layer called the "smear layer" is formed[16].

The laser has been used with importance in most medical and dental specialties, especially in Endodontics in the last decade. Its properties are known in the incision of tissues, in cases of pulpotomy, and reduction of postoperative pain. Regarding the use inside the root canal, there are still some doubts about its effectiveness. Lasers, regardless of types, are individually unable to eliminate contaminations in the root canal since the root canal temperature will reach the limit to where damage to adjacent tissues and nerves occurs before bacteria are ablated[17]. In order for lasers to ablate all contaminants in the root canal, the absorption efficiency of laser energy at the root canal surface has to increase greatly at lower laser power without substantially raising the temperature in the root canal. Due to the high affinity of water with Erbium laser, different Erbium family lasers (Er:YAG 2940nm and Er,Cr:YSGG 2780nm) represent the cutting-edge technology for laser-activated irrigant (LAI) in Endodontics. These two wavelengths are the most appropriate for different irrigant solutions, considered safe and respecting accepted clinical parameters[18].

An FDA cleared ER:Cr;YSSG laser machine (Waterlase Express™, BIOLASE®) will be tested *(clearance number: K161669, attachment 2).* This machine has different settings which are clinically suitable to be used in root canal disinfection protocols. Many studies have been published to test the efficacy of ER:Cr;YSSG laser in biofilm removal inside the root canal system[19]. Qualitative and quantitative *in vitro* studies showed that ER:Cr;YSSG laser activation of 4% NaOCl resulted in greater *E. faecalis* biofilm reduction within the dentinal tubules[20], and improved antimicrobial effect of NaOCl in comparison to syringe irrigation[21]. One blind randomized clinical trial concluded that ER:Cr;YSSG laser can achieve promising endodontic outcome, however larger sample size with longer follow-up periods are needed[22]. This study is to validate the method of using (Waterlase Express™, BIOLASE®) to ablate or disinfect microbe contaminants, and achieve cleaning of the root canal in association with standard endodontic procedures.

Proof-of-concept experiments in endodontic treatment could open the door for laser to be used in numerous clinical anti-biofilm applications. The assessment of bacterial reduction during endodontic therapy by bacterial sampling and culturing is an established technique and has been used to evaluate treatment protocols or variations thereof[23-26]. There is a current need for a better disinfecting agent that is capable of overcoming the limitations of the currently used irrigation solutions.

Based on our preliminary findings, testing local single topical application of a very low dose of Er,Cr:YSGG 2780nm (Waterlase Express™, BIOLASE®) has a potential as an advanced anti-biofilm clinical therapy with its potent antibacterial and anti-biofilm activities.

The purpose of this study is to develop an irrigation disinfection protocol for eradicating biofilms with a clinically approved and commercially available Er,Cr:YSGG 2780nm laser. This protocol will be testing local application of (Waterlase Express™, BIOLASE®), within the root canal system in patients going through routine endodontic treatment, will evaluate its potential as an advanced anti-biofilm disinfection technique and compare it to other currently used antibacterial protocols.

2.2.1 Pharmacokinetics, Pharmacodynamics and Toxicology

N/A

2.2.2 Assessment for Potential Study Products Drug-Drug, Drug-Device, Device-Device Interactions

N/A

2.2.3 Clinical Adverse Event Profile

There are no known adverse effects associated with the (Waterlase Express™, BIOLASE®) device being used in this study. The Waterlase Express™ device complies with Industry Canada license-exempt RSS standard(s) and installed, operated, and maintained according to the current revision of the guidelines specified in the Canadian standard CAN/CSA-Z386:2014 safe use of lasers in health care. It also complies with FDA laser standards. All these light sources have been independently tested by regulatory authorities and used both clinically and experimentally for over a decade. Since the device is Class IV laser, participants, providers and observers will wear appropriate laser protection eyewear (OD 3 or greater) for the 2780 nm wavelength whenever the laser is in use.

2.2.4 Dosing Rationale

Tip: RFT3, 15mJ 50Hz 0.75W 0% air 0% water, according to manufacturer instructions *(attachment 3).*

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

Only the risks or discomforts that can arise during or after any routine root canal treatment procedure apply. Bacterial sampling will not have any adverse effect on the treatment or on treatment outcome. There are no potential risks expected from the noninvasive bacteriological

sampling, including any potential physical, psychological, social, economic, monetary, legal or other risks. Identifying the antibacterial benefits of Er,Cr:YSGG laser is the goal of our experiment.

Er,Cr:YSGG laser has been used in dentistry as an alternative technology to overcome the limitations of current chemical-mechanical instrumentation techniques and improve root canal disinfection. Er,Cr:YSGG laser used in this experiment is limited within the root canal system and applied on dentine surfaces only. It will not be used systemically. 300µm laser tip will be kept within the root canal system, placed into the mid-root of the canal. The tip will be activated and slowly withdrawn to the orifice (1-2mm/sec) following the manufacturer settings as proposed in this protocol. Experiment was designed that the final root canal disinfection is not relying on be irrigated with 5cc of 3% NaOCl with the ultrasonic irrigant activation following the standard endodontic procedure protocol and then antibacterial medication ($CaOH_2$) will placed as done routinely to treat cases with pulp necrosis and apical periodontitis.

Provider, patient and assistant will wear appropriate laser protection eyewear for the 2780 nm wavelength (OD 3 or greater) whenever the laser is in use.

Data that will be collected include: demographic data, medical history/medications, tooth number, diagnosis and the PDM chart number (for potential verification of study correctness). All treatment procedures at the PDM, including the routine root canal treatment of the participants, during which the samples will be taken, are subject to strict HIPPA (privacy) and OSHA (hygiene) regulations.

2.3.2 Known Potential Benefits

The participant is not expected to get any personal benefit from taking part in this proposed research study. Microbial elimination is a key to the successful treatment of infected, necrotic teeth. Finding a better protocol to reduce intracanal infection will increase the successful outcome of endodontically treated necrotic, infected teeth and will result in an increased retention of natural teeth for the general population. The proposed study will verify by the means of microbial sampling and culturing, whether Er,Cr:YSGG laser can provide better, effective disinfection on root canal surfaces and a significant reduction of intra-canal infection *in-vivo*. If this can be demonstrated by the proposed investigation it will have clinical significance as an improvement of root canal therapy.

2.3.3 Assessment of Potential Risks and Benefits

The risks or discomforts that can arise during or after any routine root canal treatment procedure apply. All subjects will receive the same standard of care disinfection protocol, with one arm receiving the additional Waterlase Express™ laser. However, this device do involve Class IV lasers that can harm eyes. Participants, providers and observers will wear appropriate laser protection eyewear (OD 3 or greater) whenever the laser is in use. Our consent forms (for routine root canal procedures and for the study) contain clinic and investigator contact information *(attachment 4 and 5).* Subjects' concerns or complaints will be reviewed routinely.

3 STUDY OBJECTIVES AND ENDPOINTS

The purpose of this study is to develop a better, more effective disinfection protocol against biofilms within the root canal system, subsequently, improve the success of root canal treatment in teeth with apical periodontitis. This protocol will be testing local single topical application of a very low dose of 2780 nm Er,Cr:YSGG laser within the canal system in patients going through routine endodontic treatment, will evaluate its potential as an advanced antibacterial, anti-biofilm treatment by intra-canal bacterial sampling and compare it to other currently used antibacterial protocols.

OBJECTIVES	**ENDPOINTS**
Primary objective: Look at microbial colonization before and after treatment. Secondary objective: Measurment of pain, survival of the tooth, periapical bone healing and resolution of signs and symptoms.	Primary endpoint: The difference in bacterial reduction between the experimental group (laser) and the comparison group (routinely used irrigation protocol) by measuring reduction in colony forming units (CFU). Friedman's and Wilcoxon signed-rank tests will be used to compare the amount of bacteria before and after treatment in both groups, with significance levels set at 5% ($P < 0.05$). Secondary endpoints: **Measurement of pain:** Following a previous published study done at the Department of Endodontics, University of Pennsylvania[1]. Patients will be asked to rate the intensity of preoperative pain on a numeric rating scale (NRS) from 0 (no pain) to 10 (worst pain) before receiving root canal treatment. Along with NRS, the Wong-Baker facial grimace scale will also presented to the patients to help them in scoring the pain. At the end of each visit, the patients will be given a survey and asked to rate the intensity of postoperative pain at 4, 24, and 48 h after the procedure. Patients will be instructed to take 1000-mg acetaminophen as needed. If acetaminophen was consumed, the patients will be asked to record the dose and time on the survey. They will be provided stamped return envelopes to mail surveys back to the Department of Endodontics *(attachment 1).* **Treatment success determined clinically and radiographically:** Radiographically, Following periapical (PAI) index by Órstavik[2], description of radiographic fndings: 1. Normal periapical structures. 2. Small changes in the bone structure. 3. Change in the bone structure with mineral loss. 4. Periodontitis with a well-defined radiolucent area. 5. Severe periodontitis with exacerbating features.

OBJECTIVES	ENDPOINTS
	Success is defined as either complete (radiographic resolution of a periapical lesion - the radiographic sign of inflammatory processes surrounding a root tip) or incomplete healing (scar tissue formation) and failure includes uncertain healing (radiographic reduction of a periapical lesion or same lesion size) or unsatisfactory healing (increase in lesion size) as determined on the radiograph. Clinically success is defined by the absence of pain, swelling, percussion sensitivity or sinus tracts. Clinical failure is defined as the persistent presence of any of the symptoms mentioned above. Treatment success/failure measured at 6 month, 1 year and 2 year follow up (± 7 days) post root canal filling.

4 STUDY PLAN

4.1 Study Design

All the steps and procedures described are part of the standard of care root canal procedure except for sampling and the addition of Waterlase Express™ laser which are specific study procedures. All study specific procedures are **in bold** in this section.

The patient will be anesthetized, and the tooth isolated with rubber dam. Chlorhexidine 2% will be used to disinfect the tooth and the rubber dam. The removal of caries and the endodontic access will be carried out by sterile high-speed carbide burs. **Chlorhexidine 2% will be used to disinfect the tooth and the rubber dam one more time and bacteriological sample (S0) will be taken from the tooth surface.** After initial access to the root canal orifices, **a bacteriological sample will be taken from the targeted canals (S1). Sterile paper points will be placed into the canal orifices, allowed to saturate and then transferred to a vial containing reduced transport fluid (RTF).**

For NaOCl group, canals will be instrumented up to size 30/0.04 taper using 1.5cc of 3% NaOCl in between files. **For Er,Cr:YSGG laser group, canals will be instrumented up to size 30/0.04 taper using 1.5cc of normal saline in between files, then rinse each canal with 1cc normal saline and flood the access. Then ER:Cr;YSSG laser 2780nm (Waterlase Express™, BIOLASE®) with 300µm tip (EdgePro #3, RFT 3) will be placed into the mid-root of the canal. The tip will be activated and slowly withdrawn to the orifice (1-2mm/sec) following the manufacturer settings (energy 15mJ, repetition rate 50Hz, 0% air, 0% water). When the final 30.04 taper apical size is reached, a second bacterial sample will be taken (S2).**

Standard root canal procedures:

All canals in all groups will be irrigated with 5cc of 3% NaOCl with the ultrasonic irrigant activation following the standard endodontic procedure protocol. Upon completion of irrigation, **a third bacterial sample will be taken (S3). Before all samplings, solutions will be removed with 5cc of sterile saline and canal content will be neutralized with 5% sodium thiosulfate solution**. The remaining treatment sequences of the routine root canal therapy will be carried out after these procedures including further root-end enlargement and final routine irrigation protocol. The root canals will be dried with paper points, a medication (calcium hydroxide) will be placed and the teeth sealed with a temporary restoration. Patients will return after one week (± 3 days) for completion of the root filling. For both groups, the treatment procedures carried out during this investigation do not differ from the standard root canal treatment protocol with the exception of additional irrigation step with Er,Cr:YSGG laser and the bacteriologic sampling procedures.

The paper points used to take the bacteriological sampling will be transferred to the microbiology laboratory using a vial containing 1ml of reduced transport fluid (RTF). The laboratory procedures will be performed at the University of Pennsylvania Leon Levy Oral Health Sciences Building of the School of Dental Medicine in the Microbiology Laboratory (Dr. Teles' laboratory). Vial labels will contain information on tooth number, sample number (S0-S1-S2-S3) and the experimental group. Patient information will not be used in any case. RTF vials with samples will be vortexed before preparing aliquots. Samples with dilutions of 10, 100, and 1000-

fold will be prepared under anaerobic conditions using sterile glassware. Cell culture dishes with anaerobic sheep blood agar will be inoculated with 0.25 ml of undiluted sample, as well as each of the three dilutions. The culture plates will be incubated at 37°C in an anaerobic glove box containing 5% hydrogen, 10% nitrogen, and 85% CO_2 for 7 days. After incubation, the number of colony forming units will be determined by using a stereoscope. Independent t-test and Wilcoxon-Mann-Whitney test will be used for statistical analysis.

4.2 Scientific Rationale for Study Design

For standard irrigation protocol group (NaOCl/control group): canals will be instrumented up to size 30/0.04 taper using 1.5cc of 3% NaOCl in between files following the standard endodontic procedure protocol.

For Er,Cr:YSGG laser group: canals will be instrumented up to size 30/0.04 taper using 1.5cc of normal saline in between files, then rinse each canal with 1cc normal saline and flood the access. Then ER:Cr;YSSG laser 2780nm (Waterlase Express™, BIOLASE®) with 300µm tip (EdgePro #3, RFT 3) will be placed into the mid-root of the canal. The tip will be activated and slowly withdrawn to the orifice (1-2mm/sec) following the manufacturer settings (energy 15mJ, repetition rate 50Hz, 0% air, 0% water).

When the final 30.04 taper apical size is reached. All canals in all groups will be irrigated with 5cc of 3% NaOCl with the ultrasonic irrigant activation following the standard endodontic procedure protocol.

4.3 Justification for Dose

Tip: RFT3, 15mJ 50Hz 0.75W 0% air 0% water, following the manufacturer instructions *(attachment 3)*.

4.4 End of Study Definition

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Appendix Section 12.1.

5 STUDY POPULATION

5.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Provision of signed and dated informed consent form.
2. Stated willingness to comply with all study procedures and availability for the duration of the study.
3. Male or female (Gender is not an inclusion or exclusion criteria), aged 18 years old or above.
4. In good general health as evidenced by medical history or non-contributory medical history (Patient can be seen for regular dental appointment in PDM; ASA classes I and II).
5. Radiographic presence of periapical radioluceny.
6. Negative response to thermal sensitivity testing (difluordichlormethane at -50 °C, Endo-Ice, Coltène/Whaledent Inc., Cuyahoga Falls, Ohio) or electric pulp testing.
7. Enough tooth structure for adequate isolation with rubber dam.
8. No history of previous endodontic treatment on the tooth.
9. Teeth with single canal and lower molars with two mesial canals.

5.2 Exclusion Criteria

Economic status, gender, race or ethnicity are not of concern for the proposed investigation and therefore for study exclusion. The cut-off age of 18 years was only chosen so as to limit the need for parental consent. Patients will not be eligible to participate in the study if any of the following exclusion criteria applies:.

1. Patients who report they are pregnant.
2. Teeth affected by dental trauma.
3. Periodontal changes (pockets 3 mm, mobility I or gingival edema).
4. Radiographic presence of resorptive processes.
5. Per the investigator's discretion, unable or unlikely to comply with study procedure.
6. Presence of any condition which, in the opinion of the investigator, makes participation in the study not in the individual's best interest.

5.3 Lifestyle Considerations

N/A

5.4 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing

requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.5 Strategies for Recruitment and Retention

Patients may be prescreened (through medical records/phone script) and will be contacted and informed about the study ahead of time, at least 1 day before their appointment. We will try our best to contact patients and inform them about the study ahead of time, but sometimes it may not be possible. The Endodontics clinic is a referral based clinic, sometimes we see new external referrals or emergency patients. We don't have any direct contact with those patient ahead of time, and sometimes emergency patients would show up without prior appointments. Thus, patient may be included even if they haven't been contacted prior.

Patients presenting to the Department of Endodontics, School of Dental Medicine (PDM), University of Pennsylvania for evaluation and routine endodontic treatment of infected, necrotic teeth with chronic apical periodontitis will be asked to take part in the study if they appear to meet the inclusion criteria specified above. Patients will be asked if they were interested to participate in this investigation after a clinical diagnosis matching is confirmed and the patient/tooth meets the inclusion criteria specified above. Initial clinical diagnosis will be made by a postgraduate resident and verified by the supervising faculty. The PI, a full-time faculty, and the co-investigator, endodontic resident, will be informed about a potential study participant, verify the diagnosis and ask for participation (including consent and alternatives). Any recruitment materials that are developed will be submitted to the IRB for approval prior to use. No non-registered PDM patients will be asked to participate in the study.

6 STUDY INTERVENTION

6.1 Study Intervention(s) Administration

6.1.1 Study Intervention Description (attachment 3)

- **Dimensions:**

• Laser console (W x L x H) 9 x 18 x 12 in (23 x 46 x 30.5 cm)
• Tablet (W x H x D) 6.7 in x 9.3 in x 0.2 in (16.9 cm x 23.8 cm x 0.6 cm)
• Screen size 9.7 in (24.6 cm)
• Weight laser console (with water) 27.2 lbs (12.3 kg)
• Weight Tablet 0.9 lbs (390 g)

- **Electrical:**

Class I Medical Electrical (ME) Equipment
• Operating voltage 100 - 240 VAC
• Frequency 50 / 60 Hz
• Current rating 6A / 3A
• Main control Main Power Switch
• On / Off control Keyswitch
• Remote interruption Remote interlock connector

- **Air and water spray:**

• Water type Distilled or De-Ionized only
• External air source 60-120 psi. (4.1 – 8.2 bar)
• Water 0 - 100%
• Air 0 - 100%

- **Optical:**

• Laser classification IV (4)
• Medium Er,Cr:YSGG
(Erbium, Chromium: Yttrium, Scandium, Gallium, Garnet)
• Wavelength 2.78 µm (2780nm)
• Mode Free-running Pulsed
• Frequency (Pulse Rate) 5 – 50 Hz
• Average power 0.1- 4.0 W
• Power accuracy ± 20%
• Pulse energy 10– 250 mJ
• Pulse duration for “H” mode (Short pulse) 60 µs
• Pulse duration “S” mode (Long pulse) 700 µs
• Handpiece head angles 70° contra-angle
• Fiber Tip diameter range (Spot size) 200 – 1200 µm
• Output divergence ≥ 8° per side
• Aiming beam 625-670 nm (red) laser, 1mW max (Laser Class 1)
• Nominal ocular hazard distance (NOHD) 5cm
• Maximum permissible exposure (MPE) 3.46 X $10^5 W/m^2$

6.1.2 Dosing and Administration

For standard irrigation protocol group (NaOCl group): canals will be instrumented up to size 30/0.04 taper using 1.5cc of 3% NaOCl in between files following the standard endodontic procedure protocol.

For Er,Cr:YSGG laser group: canals will be instrumented up to size 30/0.04 taper using 1.5cc of normal saline in between files, then rinse each canal with 1cc normal saline and flood the access. Then ER:Cr;YSSG laser 2780nm (Waterlase Express™, BIOLASE®) with 300µm tip (EdgePro #3, RFT 3) will be placed into the mid-root of the canal. The tip will be activated and slowly withdrawn to the orifice (1-2mm/sec) following the manufacturer settings (energy 15mJ, repetition rate 50Hz, 0% air, 0% water).

When the final 30.04 taper apical size is reached. All canals in all groups will be irrigated with 5cc of 3% NaOCl with the ultrasonic irrigant activation following the standard endodontic procedure protocol.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and accountability

The study intervention and control products (both drugs and devices) are regularly available and routinely used in the Endodontic clinic, and will be provided to the investigator as needed.

6.2.2 Formulation, Appearance, Packaging, and Labeling

N/A

6.2.3 Product Storage and Stability

Control (NaOCl): Will be stored and used as regulated by the Endodontic clinic.
Waterlase Express™ and accessories will be stored in a cool dry place when not in use. Storage temperature should be 5° to 45°C (41°F to 113°F), relative humidity 10% to 90%, non-condensing. Cover the system when not in use for extended periods of time and store in a place where it will not be accidentally bumped or banged.

6.2.4 Preparation

Control (NaOCl): 12cc syringe will be filled with 3% NaOCl to be dispensed using 30 gauge needle as used routinely in the Endodontic clinic.

Waterlase Express™:

- **Daily start-up:**

• Power up the Tablet. Verify that all connections have been properly secured; turn the main power switch at the back of the console ON (I) (when the power switch is OFF, LEDs will not light up); insert the key into the Keyswitch and rotate it clockwise to the ON (I) position. The LED light on

the Tablet Holder lights up when the Tablet is properly powered on and docked in the Tablet Holder, connected and active.
• Make sure the air supply is connected and verify that the patient water bottle is no less than ⅓ full with distilled or de-ionized water. It is suggested to completely fill the patient water bottle at the start of the day.

- **Initiating the Laser From off Status:**

• Turn the main power switch at the back of the console ON (I) (when the power switch is OFF, LEDs will not light up); insert the key into the Keyswitch and rotate it clockwise to the ON (I) position
• Press and hold the power switch on the right side of the Tablet to turn it on
• The startup screen will appear, and the system software will begin to load (approximately 30 seconds)
• Verify that the Footswitch and laser are paired
• Attach the Handpiece to the Fiber Optic Cable
• Ensure the tablet is properly docked in the Tablet Holder and secured with the Tablet Latch.
• All users configured to the system listed on the User selection screen; select the proper account.
A screen will appear giving the user the option to prim the Handpiece or skip this step (if the Handpiece is already primed). If "prime" is chosen, a message will appear confirming that priming is taking place (approximately 6 seconds).
After priming, set-up is complete; the system will now transition to the home (Procedure) screen in Standby mode.

- **Activating the Waterlase Express™:**

Place the system into Ready mode after selecting a procedure (Endodontic) by pressing the Function Control Button on the Tablet Holder; wait approximately 2 seconds, then press down on the Footswitch when ready to fire the laser.

6.3 Measures to Minimize Bias: Randomization and Blinding

Simple randomization by generating simple allocation sequence, allocation concealment, and implementation of the random allocation sequence upon enrollment.

As this is part of a procedure, the investigator and study participant cannot be blinded to the treatment. To minimize bias, the investigator will provide the same information regarding reporting pain to all study participants.

6.4 Study Intervention Compliance

The study intervention will occur during a routine endodonic procedure. Administration of the study intervention will be done by qualified personnel and recorded in the case report forms.

6.5 Concomitant Therapy

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications and supplements.

6.5.1 Rescue Medicine

All participants will be provided with the Post Endodontic Treatment Instructions form *(attachment 6)*. This form will include those information regarding rescue medicine:

Take 1000-mg acetaminophen as needed. (Tylenol; 2 tablets of 500mg as needed).

If acetaminophen was consumed, the patients will be asked to record the dose and time on the survey.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

If the subject is discontinued from the study intervention, they will be withdrawn from the study. The reason for withdrawal will be recorded in the case report forms.

7.2 Participant Discontinuation/Withdrawal from the Study

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant non-compliance to attend scheduled visits
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which requires discontinuation of the study
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF). Subjects who sign the informed consent form and are randomized but do not receive the treatment may be replaced. Subjects who sign the informed consent form and are randomized and receive the treatment, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

7.3 Lost To Follow-Up

A participant will be considered lost to follow-up if he or she fails to return for 1 scheduled visit and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit (1 month) and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENT AND PROCEDURES

8.1 Efficacy Assessments

Primary objective: Look at microbial colonization before and after treatment.

The treatment and sampling procedures will largely follow the protocol described by Shuping et al.[24]. Briefly, the patient will be anesthetized, and the tooth isolated with rubber dam. Chlorhexidine 2% will be used to disinfect the tooth and the rubber dam. The removal of caries and the endodontic access will be carried out by sterile high-speed carbide burs. Chlorhexidine 2% will be used to disinfect the tooth and the rubber dam one more time and bacteriological sample (S0) will be taken from the tooth surface. After initial access to the root canal orifices, a bacteriological sample will be taken from the targeted canals (S1). Sterile paper points will be placed into the canal orifices, allowed to saturate and then transferred to a vial containing reduced transport fluid (RTF). For NaOCl group, canals will be instrumented up to size 30/0.04 taper using 1.5cc of 3% NaOCl in between files. For Er,Cr:YSGG laser group, canals will be instrumented up to size 30/0.04 taper using 1.5cc of normal saline in between files, then rinse each canal with 1cc normal saline and flood the access. Then ER:Cr;YSSG laser 2780nm (Waterlase Express™, BIOLASE®) with 300µm tip (EdgePro #3, RFT 3) will be placed into the mid-root of the canal. The tip will be activated and slowly withdrawn to the orifice (1-2mm/sec) following the manufacturer settings (energy 15mJ, repetition rate 50Hz, 0% air, 0% water). When the final 30.04 taper apical size is reached, a second bacterial sample will be taken (S2). All canals in all groups will be irrigated with 5cc of 3% NaOCl with the ultrasonic irrigant activation following the standard endodontic procedure protocol. Upon completion of irrigation, a third bacterial sample will be taken (S3). Before all samplings, solutions will be removed with 5cc of sterile saline and canal content will be neutralized with 5% sodium thiosulfate solution. The remaining treatment sequences of the routine root canal therapy will be carried out after these procedures including further root-end enlargement and final routine irrigation protocol. The root canals will be dried with paper points, a medication (calcium hydroxide) will be placed and the teeth sealed with a temporary restoration. Patients will return after one week (± 3 days) for completion of the root filling. For both groups, the treatment procedures carried out during this investigation do not differ from the standard root canal treatment protocol with the exception of additional irrigation step with the experimental solution and the bacteriologic sampling procedures.

The paper points used to take the bacteriological sampling will be transferred to the microbiology laboratory using a vial containing 1ml of reduced transport fluid (RTF). The laboratory procedures will be performed at the University of Pennsylvania Leon Levy Oral Health Sciences Building of the School of Dental Medicine in the Microbiology Laboratory (Dr. Teles' laboratory). Vial labels will contain information on tooth number, sample number (S0-S1-S2-S3) and the experimental group. Patient information will not be used in any case. RTF vials with samples will be vortexed before preparing aliquots. Samples with dilutions of 10, 100, and 1000-fold will be prepared under anaerobic conditions using sterile glassware. Cell culture dishes with anaerobic sheep blood agar will be inoculated with 0.25 ml of undiluted sample, as well as each

of the three dilutions. The culture plates will be incubated at 37°C in an anaerobic glove box containing 5% hydrogen, 10% nitrogen, and 85% CO_2 for 7 days. After incubation the number of colony forming units will be determined by using a stereoscope. Independent t-test and Wilcoxon-Mann-Whitney test will be used for statistical analysis.

Secondary objectives: Measurement of pain, survival of the tooth, periapical bone healing and resolution of signs and symptoms.

Measurement of pain: Following a previously published study conducted at the Department of Endodontics, University of Pennsylvania[1]. Patients will be asked to rate the intensity of preoperative pain on a numeric rating scale (NRS) from 0 (no pain) to 10 (worst pain) before receiving root canal treatment. Along with NRS, the Wong-Baker facial grimace scale will also presented to the patients to help them in scoring the pain. At the end of each visit, the patients will be given a survey and asked to rate the intensity of postoperative pain at 4, 24, and 48 h after the procedure. Patients will be instructed to take 1000-mg acetaminophen as needed. If acetaminophen was consumed, the patients will be asked to record the dose and time on the survey. They will be provided stamped return envelopes to mail surveys back to the Department of Endodontics *(attachment 1).*

Treatment success determined clinically and radiographically: Radiographically, Following periapical (PAI) index by Órstavik[2], description of radiographic fndings: 1. Normal periapical structures. 2. Small changes in the bone structure. 3. Change in the bone structure with mineral loss. 4. Periodontitis with a well-defined radiolucent area. 5. Severe periodontitis with exacerbating features. Success is defined as either complete (radiographic resolution of a periapical lesion - the radiographic sign of inflammatory processes surrounding a root tip) or incomplete healing (scar tissue formation) and failure includes uncertain healing (radiographic reduction of a periapical lesion or same lesion size) or unsatisfactory healing (increase in lesion size) as determined on the radiograph. Clinically, success is defined by the absence of pain, swelling, percussion sensitivity or sinus tracts. Clinical failure is defined as the persistent presence of any of the symptoms mentioned above. Treatment success/failure measured at the standard of care 6 month, 1 year and 2 year follow up (± 7 days) post root canal filling.

8.2 Safety and Other Assessments

The resident performing the endodontic treatment will evaluate and treatment plan the case as routinely performed through a clinical and radiographic examination of the involved teeth. At least one periapical radiograph of the involved tooth will be obtained during the consultation as routine protocol requires for every root canal treatment. In order to be eligible to participate in this study, an individual must meet all of the previously mentioned criteria (check section 5.1 and 5.2). On the day of procedure, the resident will conduct and complete the root canal treatment as standard protocol requires, including medical history review/update, clinical assessment, vital signs (blood pressure). Participants, providers and observers will wear appropriate laser protection eyewear (OD 3 or greater) whenever the laser is in use.

8.3 Adverse Events and Serious Adverse Events

8.3.1 Definition of Adverse Events (AE)

An adverse event (AE) is any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention related. Intercurrent illnesses or injuries should be regarded as adverse events.

A pre-existing condition should be recorded as an adverse event if the frequency, intensity or the character of the condition changes.

8.3.2 Definition of Serious Adverse Events (SAE)

Serious Adverse Events (SAE)

Adverse events are classified as serious or non-serious. A serious adverse event is any AE that, in the view of the investigator is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event when the event does not fit the other outcomes, but the event may jeopardize the patient and may require medical or surgical intervention (treatment) to prevent one of the other outcomes.
- required intervention to prevent permanent impairment or damage

Important medical events are those that may not be immediately life threatening but are clearly of major clinical significance. They may jeopardize the subject and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

8.3.3 Unanticipated Adverse Device Effect (UADE)

A UADE is any serious adverse effect on health or safety, or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

8.3.4 Classification of an Adverse Event

8.3.4.1 Severity of Event

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- Mild – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- Moderate – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

8.3.4.2 Relationship to Study Intervention

All adverse events (AEs) must have their relationship to laser assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be considered.

- Related – The AE is known to occur with the laser, there is a reasonable possibility that the laser caused the AE, or there is a temporal relationship between the laser and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the laser and the AE.
- Not Related – There is not a reasonable possibility that the administration of the laser caused the event, there is no temporal relationship between the laser and event onset, or an alternate etiology has been established.

OR

- Definitely Related – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to laser administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the laser group (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- Probably Related – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the laser, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable

response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

- Possibly Related – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the laser). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- Unlikely to be related – A clinical event, including an abnormal laboratory test result, whose temporal relationship to laser administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the laser) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- Unrelated – The AE is completely independent of laser administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.3.4.3 Expectedness

Expected adverse events include those that are expected as part of routine root canal treatment such as: the possibility of instruments breaking within the canals; perforations (extra opening) of the crown or root of the tooth; damage to existing fillings, crown or bridges; fractures of the tooth; discomfort; jaw muscle cramps and spasms; temporomandibular (jaw) joint difficulty; swelling and pain. During and after the treatment, complications may be discovered which make treatment impossible or which may require endodontic surgery or extraction of the tooth. Complications that anesthesia, injection, prescribed analgesics (pain relievers) may include, but are not limited to: swelling, infection, bleeding, discoloration of the face, discomfort, pain, nausea, drowsiness, allergic reactions, numbness or tingling of the lip, gum, or tongue (this condition is usually temporary).

A separate consent form explaining all expected adverse events for patients participating in the study will be discussed and signed by each participant *(attachment 5)*. Waterlase Express™: Water from spray may splash during laser use. This device do involve Class IV lasers that can harm eyes. Participants, providers and observers will wear appropriate laser protection eyewear (OD 3 or greater) whenever the laser is in use. No other known risks are expected when using Waterlase Express™ for root canal disinfection *(attachment 3)*.

Investigator will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described.

8.3.5 Time Period and Frequency for Event Assessment and Follow-Up

Safety will be assessed by monitoring and recording potential adverse effects using the CTCAE at each study visit. Participants will be monitored by medical histories, physical examinations. If CTCAE grading does not exist for an adverse event, the severity of mild, moderate, severe, life-threatening, and death, corresponding to Grades 1-5, will be used whenever possible.

At each contact with the subject, the investigator will seek information on adverse events by non-directive questioning and, as appropriate, by examination. Adverse events may also be detected when they are volunteered by the subject during the screening process or between visits, or through physical examination, laboratory test, or other assessments. Information on all adverse events will be recorded in the source documentation. To the extent possible, adverse events will be recorded as a diagnosis and symptoms used to make the diagnosis recorded within the diagnosis event.

As much as possible, each adverse event or follow-up information will be evaluated to determine:

1. Severity grade (CTCAE Grade 1-5)
2. Duration (start and end dates)
3. Relationship to the study treatment or process – [Reasonable possibility that AE is related: No (unrelated/ not suspected) or Yes (a suspected adverse reaction)]. If yes (suspected) - is the event possibly, probably or definitely related to the investigational treatment?
4. Expectedness to study treatment or process – [Unexpected – if the event severity and/or frequency is not described in the investigator brochure or protocol].
5. Action taken with respect to study or investigational treatment or process (none, dose adjusted, temporarily interrupted, permanently discontinued, unknown, not applicable)
6. Whether medication or therapy taken (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
7. Whether the event is serious

Once an adverse event is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study treatment, the interventions required to treat it, and the outcome.

8.3.6 Adverse Event Reporting

Reporting Period

Adverse events will be reported from the time of informed consent until study completion.

Investigator Reporting: Notifying the Study Sponsor

N/A

Investigator Reporting: Local Reporting Requirements

The investigator will report AEs and SAEs to the IRB/EC of record and other local regulatory groups per the local requirements.

New information regarding the SAE will be reported as it becomes available and in the same manner that the initial SAE (i.e. SAE form). The investigator will follow the event to resolution or until the event is deemed and documented irreversible, whichever is longer.

8.3.7 Serious Adverse Event Reporting

The study investigator shall complete an Unanticipated Adverse Device Effect Form and submit to the reviewing Institutional Review Board (IRB) . The study investigator is responsible for conducting an evaluation of an unanticipated adverse device effect and shall report the results of such evaluation to the Food and Drug Administration (FDA) and to all reviewing IRBs and participating investigators per the applicable regulation.

8.3.8 Reporting Events to Participants

N/A

8.3.9 Events of Special Interest

N/A

8.3.10 Reporting of Pregnancy

Pregnancy, in and of itself, is not regarded as an AE unless there is suspicion that study device or process may have interfered with the effectiveness of a contraceptive medication or method. When a pregnancy has been confirmed in a subject, and the fetus is exposed to study device and/or process (maternally or paternally), the following procedures should be followed to ensure subject safety:

Data on fetal outcome are collected for regulatory reporting and device safety evaluation. Follow-up should be conducted for each pregnancy to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

8.4 Unanticipated Problems

8.4.1 Definition of Unanticipated Problems (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board

(IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 Unanticipated Problem Reporting

Unanticipated problems (UPs) such as:

- Post-marketing withdrawal of a drug, device, or biologic used in a research protocol due to safety concerns.
- FDA ban of a drug, device, or biologic used in a research protocol due to safety concerns.
- Complaint of a participant when the complaint indicates unexpected risks, or the complaint cannot be resolved by the research team
- Breach of confidentiality
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study
- Premature closure of a study (e.g., due safety, lack of efficacy, feasibility, financial reasons, etc.)

should be reported by the investigator to the reviewing Institutional Review Board (IRB) and to the Data Coordinating Center (DCC)/lead principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported as any other SAE.

- Any other UP will be reported to the IRB and to the DCC/study sponsor the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) after the IRB's receipt of the report of the problem from the investigator.

8.4.3 Reporting Unanticipated Problems To Participants

N/A

8.5 Device Reporting

Safety reporting for the device will be according to 21 CFR 812.150.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

H_0: There is no difference between the laser group and the control group in microbial load reduction based on CFU.

H_a: There is a difference between the laser group and the control group in microbial load reduction based on CFU.

Primary endpoint: The difference in bacterial reduction between the experimental group (laser) and the comparison group (routinely used irrigation protocol) by measuring reduction in colony forming units (CFU). Friedman's and Wilcoxon signed-rank tests will be used to compare the amount of bacteria before and after treatment in both groups, with significance levels set at 5% ($P < 0.05$).

Secondary endpoints:

Measurement of pain: Following a previous published study done at the Department of Endodontics, University of Pennsylvania[1]. Patients will be asked to rate the intensity of preoperative pain on a numeric rating scale (NRS) from 0 (no pain) to 10 (worst pain) before receiving root canal treatment. Along with NRS, the Wong-Baker facial grimace scale will also presented to the patients to help them in scoring the pain. At the end of each visit, the patients will be given a survey and asked to rate the intensity of postoperative pain at 4, 24, and 48 h after the procedure. Patients will be instructed to take 1000-mg acetaminophen as needed. If acetaminophen was consumed, the patients will be asked to record the dose and time on the survey. They will be provided stamped return envelopes to mail surveys back to the Department of Endodontics *(attachment 1).*

Treatment success determined clinically and radiographically: Radiographically, Following periapical (PAI) index by Órstavik[2], description of radiographic fndings: 1. Normal periapical structures. 2. Small changes in the bone structure. 3. Change in the bone structure with mineral loss. 4. Periodontitis with a well-defined radiolucent area. 5. Severe periodontitis with exacerbating features. Success is defined as either complete (radiographic resolution of a periapical lesion - the radiographic sign of inflammatory processes surrounding a root tip) or incomplete healing (scar tissue formation) and failure includes uncertain healing (radiographic reduction of a periapical lesion or same lesion size) or unsatisfactory healing (increase in lesion size) as determined on the radiograph. Clinically success is defined by the absence of pain, swelling, percussion sensitivity or sinus tracts. Clinical failure is defined as the persistent presence of any of the symptoms mentioned above. Treatment success/failure measured at 6 month, 1 year and 2 year follow up (± 7 days) post root canal filling.

9.2 Sample Size Determination

Decrease in microbial load based in CFU.

Sample size calculation, with 80% power ($\beta = 0.20$), indicated a minimum sample size of 52 teeth (26 per group) to show a 5% ($\alpha = 0.05$) difference between groups. The calculations were made in the G*Power 3.1 program.

Test: Independent t test.
Two tails.
Statistical test: Means: Wilcoxon-Mann-Whitney test (two groups).
Effect size: 0.8 (large)
$\alpha = 0.05$, $\beta = 0.20$
Attrition rate of 30% was considered, indicated a minimum sample size of 76 teeth (38 per group).

9.3 Populations for Analyses

Patients presenting to the Department of Endodontics, School of Dental Medicine, University of Pennsylvania for evaluation and routine endodontic treatment of infected, necrotic teeth with chronic apical periodontitis who meet the inclusion criteria specified above.

9.4 Statistical Analyses

9.4.1 General Approach

For descriptive statistics, categorical data will be presented as present/absent or divided into groups. Continuous data will be presented as percentages, means with standard deviations, median, range.

9.4.2 Analysis of the Primary Efficacy Endpoint(s)

Primary endpoint: The difference in bacterial reduction between the experimental group (laser) and the comparison group (routinely used irrigation protocol) by measuring reduction in colony forming units (CFU). Friedman's and Wilcoxon signed-rank tests will be used to compare the

amount of bacteria before and after treatment in both groups, with significance levels set at 5% ($P < 0.05$).

9.4.3 Analysis of the Secondary Endpoint(s)

Secondary endpoint:

Measurement of pain: Following a previous published study done at the Department of Endodontics, University of Pennsylvania[1]. Patients will be asked to rate the intensity of preoperative pain on a numeric rating scale (NRS) from 0 (no pain) to 10 (worst pain) before receiving root canal treatment. Along with NRS, the Wong-Baker facial grimace scale will also presented to the patients to help them in scoring the pain. At the end of each visit, the patients will be given a survey and asked to rate the intensity of postoperative pain at 4, 24, and 48 h after the procedure. Patients will be instructed to take 1000-mg acetaminophen as needed. If acetaminophen was consumed, the patients will be asked to record the dose and time on the survey. They will be provided stamped return envelopes to mail surveys back to the Department of Endodontics *(attachment 1).*

Treatment success determined clinically and radiographically: Radiographically, Following periapical (PAI) index by Órstavik[2], description of radiographic fndings: 1. Normal periapical structures. 2. Small changes in the bone structure. 3. Change in the bone structure with mineral loss. 4. Periodontitis with a well-defined radiolucent area. 5. Severe periodontitis with exacerbating features. Success is defined as either complete (radiographic resolution of a periapical lesion - the radiographic sign of inflammatory processes surrounding a root tip) or incomplete healing (scar tissue formation) and failure includes uncertain healing (radiographic reduction of a periapical lesion or same lesion size) or unsatisfactory healing (increase in lesion size) as determined on the radiograph. Clinically success is defined by the absence of pain, swelling, percussion sensitivity or sinus tracts. Clinical failure is defined as the persistent presence of any of the symptoms mentioned above. Treatment success/failure measured at 6 month, 1 year and 2 year follow up (± 7 days) post root canal filling.

9.4.4 Safety Analyses

Primary endpoint: The difference in bacterial reduction between the experimental group (laser) and the comparison group (routinely used irrigation protocol) by measuring reduction in colony forming units (CFU). Friedman's and Wilcoxon signed-rank tests will be used to compare the amount of bacteria before and after treatment in both groups, with significance levels set at 5% ($P < 0.05$).

The resident performing the endodontic treatment will evaluate and treatment plan the case as routinely performed through a clinical and radiographic examination of the involved teeth. At least one periapical radiograph of the involved tooth will be obtained during the consultation as routine protocol requires for every root canal treatment. In order to be eligible to participate in this study, an individual must meet all of the previously mentioned criteria (check section 5.1 and 5.2). On the day of procedure, the resident will conduct and complete the root canal treatment as standard

protocol requires, including medical history review/update, clinical assessment, vital signs (blood pressure). Participants, providers and observers will wear appropriate laser protection eyewear (OD 3 or greater) whenever the laser is in use.

9.4.5 Baseline Descriptive Statistics

The baseline demographic and clinical characteristics of the study population, including age, gender, pre-op pain and endodontic diagnosis will be analyzid using descriptive statistics. Descriptive statistics will be presented as counts, proportions (%), mean and standard deviation whenever appropriate. The demographic and clinical characteristics of the study population will be analyzed using the Fisher's Exact Test. P-value of ≤ 0.05 will be considered statistically significant.

9.4.6 Planned Interim Analyses

N/A

9.4.7 Sub-Group Analyses

N/A

9.4.8 Tabulation of Individual Participant Data

N/A

9.4.9 Exploratory Analyses

N/A

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Informed Consent Process

10.1.1.1 Consent/Assent and Other Informational Documents Provided To Participants

Consent forms describing in detail the laser device, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to enrolling the subject in the study. The following consent materials are submitted with this protocol *(attachment 5)*.

10.1.1.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be

Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants will have the option of consenting in REDCap. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated by the Sponsor or the PI at any site if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) or Investigational Device Exemption (IDE) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

In terminating the study, the Sponsor and the Principal Investigator will assure that adequate consideration is given to the protection of the subjects' interests.

10.1.3 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at password-protected database. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by University of Pennsylvania School of Dental Medicine research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at password-protected database.

10.1.4 Future Use of Stored Specimens and Data

Data collected for this study will be analyzed and stored at *password-protected database*. After the study is completed, the de-identified, archived data will be transmitted to and stored at *password-protected database*, for use by other researchers including those outside of the study. Permission to transmit data to the Teles lab will be included in the informed consent.

With the participant's approval and as approved by local Institutional Review Boards (IRBs), de-identified biological samples will be stored at the Teles lab with the same goal as the sharing of data with the Teles lab. These samples could be used to research the causes of pulp disease, its complications and other conditions for which individuals with pulp disease are at increased risk, and to improve treatment. The Teles lab will also be provided with a code-link that will allow

linking the biological specimens with the phenotypic data from each participant, maintaining the blinding of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent regarding biosample storage may not be possible after the study is completed.

When the study is completed, access to study data and/or samples will be provided through the Teles lab.

10.1.5 Safety Oversight

The PI's; Dr. Bekir Karabucak (Chair of Endodontic Department), and Dr. Flavia Teles will be responsible for safety oversight.

10.1.6 Clinical Monitoring

Dr. Bekir Karabucak (Chair of Endodontic Department)

10.1.7 Quality Assurance and Quality Control

All monitoring and audits are to be performed according to ICH GCP E6(R2).

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated, and specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.8 Data Handling and Record Keeping

10.1.8.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data and follow ALCOAC standards (attributable, legible, contemporaneous, original, accurate, and complete).

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered in Axium and red cap, a 21 CFR Part 11-compliant data capture system provided by the Axium and red cap. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.8.2 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonization (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the Waterlase Express device. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.1.9 Protocol Deviations

The PI and the study team should document all scenarios where the protocol is not followed and provide, in particular:

- Who deviated from the protocol
- What was the deviation
- When did the deviation occur
- How did the deviation happen
- What is the impact of the deviation
- A root cause analysis of why the deviation occurred

If the assessment results in a determination that any of the following are potentially affected, the deviation would be considered of significant impact:

- having the potential to adversely affect subject safety; OR

- increases risks to participants; OR
- adversely affects the integrity of the data; OR
- violates the rights and welfare of participants, OR
- affects the subject's willingness to participate in research.
- there is a potential for an overall impact on the research that should be shared with the IRB for consideration and development of next best steps to address it

10.1.10 Publication and Data Sharing Policy

This study will comply with the data sharing agreement.

The Sponsor must approve all sharing of information/data prior to its occurrence.

10.1.11 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial.

10.2 Additional Considerations

N/A

10.3 Protocol Amendment History

Version	Date	Description of Change	Brief Rationale

Version	Date	Description of Change	Brief Rationale

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12 APPENDIX

12.1 Schedule of Activities (SoA)

Procedures	Pre-Screening Up to Day 0	Enrollment/Baseline Visit 1, Day 0	Study Visit 2 Day 7 +/-3 days	Study Visit 3 6 months +/-7 days	Study Visit 4 1 year +/-7 days	Study Visit 5 2 yeras +/-7 days
Prescreening (medical records, phone script)	X					
Study informed consent		X				
Demographics		X				
Medical history review/update		X	X	X	X	X
Clinical assessment		X	X	X	X	X
Vital signs (Blood pressure)		X	X	X	X	X
Randomization		X				
Administer study intervention		X				
Sample collection		X				
Pain Assessments (pre-op, and 4, 24, and 48 hours post-op)		X				
Adverse event review and evaluation		X	X	X	X	X
Radiologic/Imaging assessment		X	X	X	X	X
Completion of the study						X

END OF DOCUMENT